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Effect of linseed oil and fish oil alone or as an equal mixture on ruminal fatty acid metabolism in growing steers fed maize silage-based diets[1]

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ABSTRACT: Because of the potential benefits to human health, there is interest in increasing 18:3n-3, 20:5n-3, 22:6n-6, and *cis*-9,*trans*-11 CLA in ruminant foods. Four Aberdeen Angus steers (406 ± 8.2 kg of BW) fitted with ruminal and duodenal cannulas were used in a 4 × 4 Latin square experiment with 21-d periods to examine the potential of fish oil (FO) and linseed oil (LO) in the diet to increase ruminal outflow of *trans*-11 18:1 and total n-3 PUFA in growing cattle. Treatments consisted of a control diet (60:40; forage:concentrate ratio, on a DM basis, respectively) based on maize silage, or the same basal ration containing 30 g/kg of DM of FO, LO, or a mixture (1:1, wt/wt) of FO and LO (LFO). Diets were offered as total mixed rations and fed at a rate of 85 g of DM/(kg of $BW^{0.75}$/d). Oils had no effect ($P = 0.52$) on DMI. Linseed oil had no effect ($P > 0.05$) on ruminal pH or VFA concentrations, whereas FO shifted rumen fermentation toward propionate at the expense of acetate. Compared with the control, LO increased ($P < 0.05$) 18:0, *cis* 18:1 (Δ9, 12–15), *trans* 18:1 (Δ4–9, 11–16), *trans* 18:2, geometric isomers of Δ9,11, Δ11,13, and Δ13,15 CLA, *trans*-8,*cis*-10 CLA, *trans*-10,*trans*-12 CLA, *trans*-12,*trans*-14 CLA, and 18:3n-3 flow at the duodenum. Inclusion of FO in the diet resulted in greater ($P < 0.05$) flows of *cis*-9 16:1, *trans* 16:1 (Δ6–13), *cis* 18:1 (Δ9, 11, and 13), *trans* 18:1 (Δ6–15), *trans* 18:2, 20:5n-3, 22:5n-3, and 22:6n-3, and decreased ($P < 0.001$) 18:0 at the duodenum relative to the control. For most fatty acids at the duodenum, responses to LFO were intermediate of FO and LO. However, LFO resulted in greater ($P = 0.04$) flows of total *trans* 18:1 than LO and increased ($P < 0.01$) *trans*-6 16:1 and *trans*-12 18:1 at the duodenum compared with FO or LO. Biohydrogenation of *cis*-9 18:1 and 18:2n-6 in the rumen was independent of treatment, but both FO and LO increased ($P < 0.001$) the extent of 18:3n-3 biohydrogenation compared with the control. Ruminal 18:3n-3 biohydrogenation was greater ($P < 0.001$) for LO and LFO than FO, whereas biohydrogenation of 20:5n-3 and 22:6n-3 in the rumen was marginally less ($P = 0.05$) for LFO than FO. In conclusion, LO and FO at 30 g/kg of DM altered the biohydrogenation of unsaturated fatty acids in the rumen, causing an increase in the flow of specific intermediates at the duodenum, but the potential of these oils fed alone or as a mixture to increase n-3 PUFA at the duodenum in cattle appears limited.

Key words: biohydrogenation, cattle, conjugated linoleic acid, fish oil, linseed oil, *trans* fatty acid

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INTRODUCTION

Public health guidelines in most developed countries have recommended population-wide decreases in saturated and *trans* fatty acids and an increase in 18:3n-3, 20:5n-3, and 22:6n-3 in the human food chain to reduce the incidence of chronic disease (World Health Organization, 2003). Studies in human cell lines and rodents have provided evidence that *cis*-9,*trans*-11 CLA exhibits anticarcinogenic activity (Wahle et al., 2004). Ruminant-derived foods provide a significant amount of fat and represent the major source of *cis*-9,*trans*-11 CLA in the human diet (Givens and Shingfield, 2004), highlighting the potential of altering the composition of meat and milk to improve human health.

Most of the *cis*-9,*trans*-11 CLA in ruminant milk and tissue lipids is formed by the desaturation of *trans*-11 18:1 (Griinari et al., 2000; Palmquist et al., 2005). In cattle, linseed oil (**LO**) in the diet increases *trans*-11 18:1, *cis*-9,*trans*-11 CLA, and 18:3n-3 at the duodenum (Loor et al., 2004; Doreau et al., 2009b), whereas fish oil (**FO**) results in greater flows of *trans*-11 18:1, 20:5n-3, and 22:6n-3 (Shingfield et al., 2003; Kim et al., 2008; Lee et al., 2008). However, increases in n-3 PUFA at the small intestine to LO and FO are marginal due to extensive biohydrogenation in the rumen. Both 18:2n-6 and 18:3n-3 decreased the biohydrogenation of 20:5n-3 and 22:6n-3 and increased *trans*-11 18:1 accumulation in vitro (Chow et al., 2004; Wąsowska et al., 2006; Boeckaert et al., 2007), suggesting that a mixture of LO and FO may result in greater ruminal escape of 18:3n-3, 20:5n-3, and 22:6n-3 and may increase the availability of 18:1, 18:2, and CLA biohydrogenation intermediates for absorption, compared with iso-energetic amounts of either oil offered alone. To test this hypothesis, the effect of LO, FO, or a mixture (1:1, wt/wt) of both (**LFO**) on ruminal biohydrogenation and flows of fatty acids at the duodenum was examined in growing steers (*Bos p. taurus*) fed maize (*Zea mays*) silage-based diets.

MATERIALS AND METHODS

All experimental procedures were licensed, regulated, and inspected by the UK Home Office under the Animals (Scientific Procedures) Act of 1986.

Animals, Management, and Experimental Design

Four Aberdeen Angus steers of mean 406 ± 8.2 kg of BW at the start of the experiment fitted with a rumen cannula (i.d., 40 mm) and a simple T-piece cannula (i.d., 20 mm) located in the proximal duodenum within 50 mm of the pylorus (Beever et al., 1978) were used in a 4 × 4 Latin square design with 21-d experimental periods. Steers were housed in individual tie stalls within a dedicated metabolism unit and offered daily rations as equal meals at 0600 and 1800 h. Animals had continuous access to fresh water and trace-mineralized salt blocks (Baby Red Rockies, Winsford, Cheshire, UK). Steers were weighed at the beginning of the experiment and at the end of each experimental week at 1200 h. At the end of the 84 d, experiment steers weighed 512 ± 10.3 kg.

Experimental Diets

Steers were offered total mixed rations (Table 1) based on maize silage (forage:concentrate ratio 60:40, on a DM basis) at a rate of 85 g of DM/(kg of $BW^{0.75}$/d) equivalent to 95% of ad libitum intake measured at the start of the experiment. Treatments were composed of the basal diet containing no additional oil (control), 30 g/kg of DM of refined herring (*Clupea* spp.) and mackerel (*Scomber* spp.) oil (**FO**; Napro Pharma, AS, Brattvaag, Norway), *Linum usitatissimum* (**LO**; Animal Feeds Ltd., Raglan, UK), or a mixture (1:1, wt/wt) of LO and FO (**LFO**; Table 1). Diets were fed as total mixed rations at a restricted intake to avoid selection of dietary components and maintain a constant forage:concentrate ratio across treatments. Concentrates were formulated according to Agricultural and Food Research Council (1993) to meet the nutrient requirements of growing cattle. Ration mixes were adjusted weekly for changes in component DM content. Oil supplements were mixed with concentrate ingredients immediately before the addition of maize silage to optimize oil dispersal in the diet. Daily feed rations were offered as equal meals at 0600 and 1800 h. At the end of each week, the amount of total mixed ration prepared for each steer was adjusted according to measurements of BW. Feed refusals were collected and weighed at 0545 h on each day of the experiment. Forage maize (*cv.* Hudson) was harvested using a forage harvester fitted with a kernel processor and ensiled directly without additive.

Measurements, Sample Collection, and Chemical Analysis

Individual animal intakes were recorded daily, but only measurements on d 16 to 21 of each experimental period were used for statistical analysis. During the last 5 d of each experimental period, samples of fresh maize silage, concentrate ingredients, and feed refusals were collected daily, and DM content was determined by drying in a forced-air oven at 100°C for 24 h. Feed samples collected daily were added to a composite sample for each experimental period and stored at −20°C. Frozen samples of maize silage were analyzed for VFA, ethanol, lactic acid, and ammonia N using accredited and parliamentary-approved procedures for feedstuff analysis (Statutory Instruments, 1982, 1985) by a commercial laboratory (Natural Resources Management, Bracknell, UK) and used to correct the DM content of maize silage for volatile losses during drying (Juniper et al., 2005). Organic matter content of maize silage and concentrates was determined by ashing at 550°C for

Table 1. Ingredient and chemical composition of experimental diets

Item	Treatment[1]			
	Control	LO	FO	LFO
Ingredient, g/kg of DM				
Maize silage	600	600	600	600
Soybean (*Glycine max*) meal	150	138	138	138
Rapeseed (*Brassica napus*) meal[2]	150	138	138	138
Wheat (*Triticum* spp.) middlings	62.5	57.6	57.6	57.6
Linseed oil	0	30.0	0	15.0
Fish oil	0	0	30.0	15.0
Blended cane molasses and urea[3]	12.5	11.5	11.5	11.5
Minerals and vitamins[4]	15.6	14.4	14.4	14.4
Limestone	9.4	8.6	8.6	8.6
Chemical composition, g/kg of DM				
OM	908	912	912	912
NDF	286	280	280	280
Starch	222	221	221	221
CP	208	196	196	196
Water-soluble carbohydrates	46.1	42.9	42.9	42.8
14:0	0.1	0.1	2.1	1.1
16:0	4.2	5.2	8.3	6.7
16:1 *cis*-9	0.1	0.1	2.3	1.2
18:0	0.6	1.4	1.3	1.3
18:1 *cis*-9	6.3	10.7	9.2	9.9
18:1 *cis*-11	0.9	1.0	1.6	1.3
18:2n-6	10.5	14.7	10.6	12.6
18:3n-3	1.3	17.6	1.5	9.6
18:4n-3	0.0	0.0	0.8	0.4
20:5n-3	0.0	0.0	4.7	2.3
22:5n-3	0.0	0.0	0.5	0.2
22:6n-3	0.0	0.0	3.0	1.5
Total fatty acids	26.1	53.4	53.4	53.3

[1]Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (LFO).

[2]Solvent-extracted rapeseed meal of low glucosinolate content.

[3]Regumaize 44 (SvG Intermol Limited, Bootle, Merseyside, UK); declared composition (g/kg of DM): CP (440), water-soluble carbohydrates (550), and ME content (11.8 MJ/kg of DM).

[4]Proprietary mineral supplement (Dairy Direct, Bury St. Edmonds, UK) declared as containing (g/kg) calcium (270), magnesium (60), sodium (40), phosphorus (40), zinc (5.0), manganese (4.0), copper (1.5); (mg/kg), iodine (500), cobalt (50), selenium (15), retinyl acetate (150), cholecalciferol (2.50), and DL-α-tocopheryl acetate (500).

16 h. Neutral detergent fiber concentrations in maize silage and concentrates corrected for residual ash were measured in the presence of SDS and α-amylase using an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). Feed starch content was measured using the amyloglucosidase technique followed by the determination of total reducing substances and correction for water-soluble carbohydrates. Measurements of N content were determined using the Kjeldahl technique, and water-soluble carbohydrate content was assessed using spectrophotometry (Juniper et al., 2005). Samples (40 mL) of rumen fluid (n = 9) were collected from the ventral sac on d 20 of each period from each steer at 1.5-h intervals starting at 0600 h. After removal, pH was measured (pH meter HI8520; Hanna Instruments Ltd., Leighton Buzzard, UK) and samples were stored at −20°C. At the end of the experiment, samples of rumen fluid collected at each time point were pooled on an equal volume basis, and daily composite samples were analyzed for VFA by a commercial laboratory (Natural Resources Management) using the same procedures as for feeds.

Digesta flow was determined using LiCo-EDTA and Cr-mordanted straw as indigestible markers for liquid and particulate phases, respectively (Ahvenjärvi et al., 2003). Coarsely chopped barley straw was soaked in tap water overnight, rinsed with neutral detergent, and labeled with chromium (Udén et al., 1980). The Cr-mordanted straw containing 40.5 ± 0.30 mg of Cr/g of DM was administered (20 g/d) twice daily on top of the rumen contents via the cannula at 12-h intervals starting at 1800 h on d 14 of each experimental period. The LiCo-EDTA (6 g), prepared according to standard procedures (Udén et al., 1980), was dissolved in 3 L of distilled water and infused at 1800 h on d 14 into the rumen at a constant rate (2.1 mL/min). Ruminal infusions were performed using polyamide tubing (internal diameter 4 mm) that passed through the rumen fistula connected to a peristaltic pump (model 202, Watson-Marlow, High Wycombe, UK). Markers were adminis-

tered to each animal to provide daily doses of 0.9 and 0.8 g/d of Co and Cr, respectively. At the start of each marker administration, steers were given priming doses of LiCo-EDTA and Cr-mordanted straw supplying 1.35 and 1.0 g of Co and Cr, respectively, to facilitate rapid equilibration of the marker concentrations in the rumen.

Spot samples (250 mL) of digesta at the duodenum were collected 3 times daily at 4-h intervals over the last 4 d of each experimental period starting at 0600 h on d 18. Immediately after collection, 2.5 mL of 2, 6-ditert-butyl-4-methoxyphenol in 80% (vol/vol) methanol (1 mg/mL) was added to prevent the oxidation of NEFA, and samples were stored under N_2 at –20°C. At the end of the study, digesta from each animal was thawed at room temperature and pooled on an equal volume basis across sampling times to provide a composite sample for each experimental period. Composite digesta samples were stirred vigorously and split into 2 equal subsamples. One subsample was frozen and lyophilized as whole duodenal digesta, whereas the remainder was separated into liquid and solid phases by centrifugation at 200 × *g* for 10 min at 4°C. The supernatant was decanted and stored at –20°C, whereas the solid phase was frozen immediately, lyophilized, and stored at –20°C. Samples of solid and whole digesta were analyzed for DM, OM, N, ammonia-N, starch, and NDF using the same methods used for feed ingredients. Concentrations of Co and Cr in digesta were measured by atomic absorption spectroscopy (SpectrAA-10 analyzer, Varian Limited, Walton-On-Thames, UK) according to standard procedures (Williams et al., 1962) using samples of duodenal digesta and feces collected from one steer before the start of the experiment for calibration purposes.

Whole-tract apparent digestibility coefficients were determined by total fecal collection. Feces were collected over 120 h starting at 1000 h on d 17 of each experimental period. Total feces excreted were weighed, thoroughly mixed, subsampled (10%, wt/wt), composited for each animal within experimental period, and stored at –20°C until analyzed for DM, OM, NDF, starch, N, Cr, and Co content using the same laboratory methods used for the analysis of duodenal digesta. Flows of digesta at the duodenum were calculated after mathematical reconstitution of true digesta (Ahvenjärvi et al., 2003) based on marker excretion in feces.

Lipid Analysis

Fatty acid methyl esters (**FAME**) of lipid in FO, LO, and freeze-dried samples of maize silage and concentrates were prepared in a 1-step extraction-transesterification procedure using toluene (Sukhija and Palmquist, 1988) and 2% (vol/vol) sulfuric acid in methanol (Shingfield et al., 2003). Feed fatty acid content was determined using trinonadecanoin (T-165, Nu-Chek Prep, Elysian, MN) as an internal standard (Shingfield et al., 2010b). Lipid in solid and whole digesta was extracted in triplicate using a mixture of chloroform and methanol (2:1; vol/vol), and the organic extracts were converted to FAME by incubation with freshly prepared 0.5 *M* sodium methoxide in methanol at 50°C for 15 min followed by reaction with 5% (vol/vol) hydrochloric acid in methanol at 50°C for 60 min (Kramer and Zhou, 2001). Fatty acid content of duodenal digesta was determined using heneicosanoate in chloroform (15 mg/mL) as an internal standard (Lee et al., 2005).

The FAME were separated and quantified using a gas chromatograph (3800 CP, Varian Instruments, Walnut Creek, CA) equipped with a flame-ionization detector, automatic injector, split injection port, and a 100-m fused silica capillary column (CP-SIL 88 for FAME, Chrompack, Middelburg, the Netherlands) with helium as the carrier gas and hydrogen as the fuel gas. Total FAME profile in a 1-μL sample at a split ratio of 1:30 was determined using a temperature gradient program (Lee et al., 2005). Peaks were identified by comparison of retention times with authentic FAME standards (ME61, Larodan fine chemicals, Malmo, Sweden; S37, Supelco, Poole, Dorset, UK). Methyl esters in feed ingredients and duodenal digesta not contained in commercially available standards were formally identified by gas chromatography-mass spectrometry (**GC-MS**) analysis of 4,4-dimethyloxazoline fatty acid (**DMOX**) derivatives prepared from selected samples of FAME by incubation overnight with 2-amino,2-methyl-1-propanol under N_2 at 150°C (Shingfield et al., 2006). Impact ionization spectra of DMOX derivatives were recorded under an ionization energy of 70 eV using a GC (model 6890, Hewlett-Packard, Wilmington, DE) equipped with a selective quadrupole mass detector (model 5973N, Agilent Technologies Inc., Wilmington, DE) and a 100-m fused-silica capillary column (i.d., 0.25 mm) coated with 0.2-μm film of cyanopropyl polysiloxane (CP-SIL 88, Chrompack 7489). Analysis of DMOX derivatives was achieved using a temperature gradient and helium as the carrier gas (Shingfield et al., 2006), and the position of double bonds was located according to published guidelines (Spitzer, 1996; Christie, 1998).

Samples of FAME were evaporated under N_2, dissolved in heptane, and analyzed for CLA methyl ester composition by HPLC using 4 silver-impregnated silica columns (ChromSpher 5 lipids, 250 × 4.6 mm; 5 μm particle size, Varian Ltd., Walton-on-Thames, UK) coupled in series and 0.1% (vol/vol) acetonitrile in heptane as the mobile phase (Shingfield et al., 2003). Isomers were identified using an authentic CLA methyl ester standard (O-5632, Sigma-Aldrich, St. Louis, MO) and chemically synthesized *trans*-9,*cis*-11 CLA (Shingfield et al., 2005). Concentrations of CLA isomers in digesta were calculated based on proportionate peak area responses determined by HPLC and the sum of *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA, and *cis*-9,*trans*-11 CLA weight percentage determined by GC analysis.

Statistical Analysis

Experimental data were subject to ANOVA for a 4 × 4 Latin square using the MIXED procedure (SAS Institute Inc., Cary, NC). The statistical model included the random effects of animal, fixed effects of treatment and period, and residual error. Least squares means are reported, and treatment effects were considered significant at $P < 0.05$. Differences between diets were evaluated using the Tukey multiple comparison test.

RESULTS

Feed Composition

Maize silage had the following chemical composition (g/kg of DM, unless otherwise stated): DM (g/kg) 441, OM 932, CP 85.9, NDF 345, starch 339, pH 3.84, lactic acid 48.9, total VFA 8.91, ethanol 4.80, water-soluble carbohydrates 5.07, and ammonium N (g/kg of total N) 131. The basal concentrate had a DM content of 881 g/kg and contained (g/kg of DM), OM 874, CP 388, NDF 200, starch 50.0, and water-soluble carbohydrates 107. Measurements of fatty acid composition indicated that maize silage and concentrates contained relatively high proportions of *cis*-9 18:1 and 18:2n-6, 18:3n-3 predominated in LO, and FO was rich in 16:0, 20:5n-3, and 22:6n-3 (Table 2). Fish oil also contained significant concentrations of fatty acids not detected in the other feeds including 18:4n-3, 20:4n-6, 20:4n-6, and 22:5n-3. Linseed oil was devoid of *trans* 18:1 fatty acids, whereas trace amounts were detected in FO, maize silage, and concentrates (Table 2).

Nutrient Intake

Oil supplements had no effect ($P > 0.05$) on DM, OM, NDF, CP, starch, and water-soluble carbohydrate intake, but increased ($P < 0.01$) saturated, monounsaturated, polyunsaturated, and total fatty acid ingestion (Table 3). Relative to the control, LO in the diet increased ($P < 0.05$) 18:0, *cis*-9 18:1, 18:2n-6, 18:3n-3, and 22:0 intake, whereas FO resulted in greater ($P < 0.01$) ingestion of 12:0, 14:0, 16:0, *cis*-9 16:1, 18:0, *cis*-9 18:1, *cis*-11 18:1, *trans* 18:1, *cis*-9 20:1, *cis*-11 20:1, *cis*-13 22:1, 20:5n-3, 22:5n-3, and 22:6n-3 (Table 3). Fish oil also resulted in the consumption of several fatty acids specific to this oil source including 16:2n-4, 16:4n-1, 18:4n-3, 20:4n-3, and 21:5n-3. By design, the intake of fatty acids on the LFO treatment were intermediate relative to FO and LO (Table 3).

Rumen Fermentation

Oils in the diet had no effect ($P = 0.71$) on rumen pH or on total VFA concentrations ($P = 0.58$), but FO modified rumen fermentation, causing a decrease ($P = 0.001$) in the molar acetate:propionate ratio (Table 4). Even though molar proportions of acetate on the LFO treatment were comparable with the control and LO treatments, the ratio of acetate:propionate exhibited a similar trend as exemplified when FO was fed alone. In contrast, LO had no effect ($P > 0.05$) on rumen fermentation characteristics relative to the control (Table 4). Changes in molar VFA proportions to LFO were intermediate of the responses to FO and LO.

Nutrient Flow at the Duodenum

Dietary lipid supplements had no effect ($P < 0.05$) on the flow of DM, OM, NDF, or starch at the duodenum (Table 5). However, FO tended to reduce ($P = 0.08$) N flow and decreased ($P = 0.050$) nonammonia N at the duodenum compared with the control or LO treatment (Table 5). Relative to the control, oil supplements increased ($P < 0.001$) total fatty acid flow and altered the relative abundance of biohydrogenation intermediates and end products at the duodenum (Table 5).

Inclusion of LO in the diet increased ($P < 0.05$) 16:0, 3,7,11,15-tetramethyl 16:0, 18:0, *cis* 18:1, *trans* 18:1, CLA, and 18:3n-3 at the duodenum, whereas FO increased ($P < 0.05$) the flow of 14:0, 15:0, 15:0 *iso*, 16:0, 3,7,11,15-tetramethyl 16:0, total 16:1, 17:0, *cis* 18:1, *trans* 18:1, 20- (with the exception of 20:0 and 20:4n-6), and 22- (other than 22:0) carbon fatty acids, but decreased ($P < 0.001$) 18:0 at the duodenum (Table 5). Alterations in the flow of fatty acids on the LFO diet were in most cases intermediate of those to FO and LO (Table 5).

Supplements of LO had no effect ($P = 0.91$) on the flow of total 16:1 fatty acids compared with the control. In contrast, FO alone or when fed as a mixture with LO increased ($P < 0.01$) or resulted in the appearance of *cis*-9 16:1 and *trans*-16:1 (Δ6–13) at the duodenum (Table 6). Inclusion of oils in the diet also resulted in isomer-dependent changes in the relative proportions of *trans* 18:1 isomers in duodenal digesta and altered the flow of *cis* 18:1 and *trans* 18:1 at the duodenum (Table 6). Compared with the control, LO decreased ($P = 0.011$) the relative abundance of *trans*-6–9 (11.0 and 9.08%, respectively), whereas FO increased ($P = 0.019$) *trans*-10 (8.32 vs. 17.4%) and decreased ($P < 0.001$) the abundance of *trans*-5–8 (7.14 vs. 4.75%) and ($P = 0.005$) *trans* 13–16 (16.8 vs. 6.34%) when expressed as a percentage of total *trans* 18:1 in duodenal digesta. Treatments had no effect ($P = 0.32$) on the relative amounts of *trans*-11 in total *trans* 18:1 in digesta (mean 60.3%). Inclusion of LO in the diet increased ($P < 0.05$) duodenal *cis*-9, *cis*-12–15 18:1 and *trans*-4–9 and *trans*-11–16 18:1 flow, whereas FO in the diet increased ($P < 0.05$) the amount of *cis*-9, *cis*-11, and *cis*-13 18:1 and *trans*-6–15 18:1 at the duodenum (Table 6). Increases in the flow of most *cis* and *trans* 18:1 isomers to LFO were intermediate or comparable with the changes to LO and FO, other than an increase ($P = 0.001$) in the amount of *trans*-12 18:1 at the duodenum (Table 6).

Table 2. Fatty acid composition of experimental maize silage, basal concentrate, fish oil, and linseed oil

Fatty acid, g/100 g	Maize silage	Concentrates	Fish oil	Linseed oil
12:0	0.18	0.01	0.13	0.00
14:0	0.47	0.15	7.27	0.03
14:1 *cis*-9	0.00	0.00	0.06	0.00
15:0	0.07	0.12	0.05	0.02
16:0	16.6	15.2	15.0	4.23
16:1 *cis*-9	0.31	0.64	7.84	0.00
16:2n-4	0.00	0.00	1.15	0.00
16:4n-1	0.00	0.00	2.47	0.00
16:4n-3	0.00	0.00	0.16	0.00
17:0	0.18	0.14	0.41	0.06
18:0	2.31	2.26	2.60	2.74
18:1 *cis*-9	20.6	28.7	11.0	16.5
18:1 *cis*-11	0.81	6.59	2.71	0.62
18:1 *cis*-12	0.00	0.00	0.06	0.00
18:1 *trans*[1]	0.02	0.51	1.42	0.00
18:2 *trans*[2]	0.14	0.50	0.00	0.05
18:2n-4	0.00	0.00	0.37	0.00
18:2n-6	48.6	30.6	1.17	15.8
18:2n-9	0.00	0.00	0.38	0.00
18:3n-3	5.55	4.41	0.91	57.8
18:3n-6	0.00	0.00	0.25	0.00
18:4n-3	0.00	0.00	2.95	0.00
20:0	1.83	0.38	0.17	0.12
20:1 *cis*-9	0.18	0.06	0.16	0.04
20:1 *cis*-11	0.21	0.53	1.25	0.19
20:1 *cis*-13	0.00	0.00	0.24	0.00
20:2n-3	0.00	0.00	0.05	0.00
20:2n-6	0.15	0.17	0.00	0.01
20:2n-9	0.00	0.00	0.19	0.00
20:3n-6	0.00	0.00	0.15	0.00
20:4n-3	0.00	0.00	0.72	0.00
20:4n-6	0.00	0.00	0.79	0.00
20:5n-3	0.00	0.00	16.5	0.00
21:5n-3	0.00	0.00	0.69	0.00
22:0	0.58	0.40	0.07	0.12
22:1 *cis*-11	0.00	0.00	0.72	0.00
22:1 *cis*-13	0.10	0.17	0.24	0.01
22:5n-3	0.00	0.00	1.76	0.00
22:5n-6	0.00	0.00	0.25	0.00
22:6n-3	0.00	0.00	10.5	0.00
24:0	0.00	6.85	0.03	0.08
24:1 *cis*-15	0.00	0.14	0.46	0.01
26:0	0.20	0.10	0.00	0.03
28:0	0.11	0.04	0.00	0.00
Other	0.54	1.58	3.72	0.16
Total SFA	22.9	25.9	28.1	7.55
Total MUFA	22.2	38.1	28.7	17.7
Total PUFA	54.4	35.6	43.2	74.8
Total fatty acids, g/kg of DM	23.8	29.4	950	953

[1]Sum of *trans*-9 18:1, *trans*-10 18:1, and *trans*-11 18:1.

[2]Sum of *cis*-9,*trans*-12 18:2, *trans*-9,*cis*-12 18:2, and *trans*-9,*trans*-12 18:2.

Compared with the control diet, oil supplements altered the flow of 18:2 biohydrogenation intermediates at the duodenum. Linseed oil increased ($P < 0.05$) the amount or resulted in the occurrence of *trans*-11,*cis*-15 18:2, *trans*-9,*trans*-12 18:2, geometric isomers of Δ9,11, Δ11,13, and Δ13,15 CLA, *trans*-8,*cis*-10 CLA, *trans*-10,*trans*-12 CLA, and *trans*-12*trans*-14 CLA (Table 7). Inclusion of FO in the diet increased ($P = 0.001$) *trans*-11,*cis*-15 18:2 and ($P = 0.003$) *trans*-9,*trans*-12 18:2 at the duodenum, but had no effect ($P > 0.05$) on the flow of CLA isomers (Table 7). Increases in the flow of nonconjugated 18:2 at the duodenum to LFO relative to the control were comparable with LO, whereas changes in the flow of CLA intermediates on the LFO treatment were intermediate of the responses to FO and LO for certain isomers, but for others were more comparable with FO than LO (Table 7).

Table 3. Effect of linseed oil and fish oil in the diet on nutrient intake in growing cattle

	Treatment[1]					
Intake, kg/d	Control	LO	FO	LFO	SEM	*P*-value
Silage DM	5.28	5.27	4.96	5.29	0.176	0.52
Total DM	8.88	8.83	8.34	8.83	0.280	0.54
OM	8.06	8.05	7.61	8.05	0.255	0.56
NDF	2.53	2.47	2.33	2.47	0.077	0.37
Starch	1.97	1.96	1.85	1.96	0.064	0.51
CP	1.85	1.85	1.85	1.85	0.051	0.14
Water-soluble carbohydrates	0.41	0.38	0.36	0.38	0.011	0.09
Fatty acid,[2] g/d						
12:0	0.2^{c}	0.2^{c}	0.5^{a}	0.4^{b}	0.02	<0.001
14:0	0.7^{c}	0.8^{c}	17.8^{a}	9.7^{b}	0.75	<0.001
16:0	36.9^{d}	46.0^{c}	69.1^{a}	59.3^{b}	2.39	<0.001
16:1 *cis*-9	1.1^{c}	1.0^{c}	19.5^{a}	10.7^{b}	0.84	<0.001
16:2n-4	0.0^{c}	0.0^{c}	2.7^{a}	1.4^{b}	0.12	<0.001
16:4n-1	0.0^{c}	0.0^{c}	5.8^{a}	3.1^{b}	0.25	<0.001
18:0	5.3^{b}	11.9^{a}	11.0^{a}	11.7^{a}	0.36	<0.001
18:1 *cis*-9	56.2^{c}	94.5^{a}	77.0^{b}	87.5^{a}	2.38	0.001
18:1 *cis*-11	8.0^{c}	8.9^{c}	13.4^{a}	11.4^{b}	0.48	<0.001
18:1 total *trans*	0.4^{c}	0.4^{c}	3.7^{a}	2.1^{b}	0.16	<0.001
18:2n-6	93.2^{c}	130^{a}	88.3^{c}	111^{b}	2.72	0.001
18:3n-3	11.6^{c}	155^{a}	12.8^{c}	84.0^{b}	2.67	<0.001
18:3n-6	0.0^{c}	0.0^{c}	0.6^{a}	0.3^{b}	0.03	<0.001
18:4n-3	0.0^{c}	0.0^{c}	7.0^{a}	3.6^{b}	0.30	<0.001
20:0	2.7	3.0	3.0	3.0	0.10	0.18
20:1 *cis*-9	0.3^{d}	0.4^{c}	0.6^{a}	0.5^{b}	0.03	<0.001
20:1 *cis*-11	0.8^{c}	1.3^{c}	3.7^{a}	2.6^{b}	0.15	<0.001
20:1 *cis*-13+14+15	0.0^{c}	0.0^{c}	0.6^{a}	0.3^{b}	0.03	<0.001
20:2n-9	0.0^{c}	0.0^{c}	0.5^{a}	0.2^{b}	0.02	<0.001
20:3n-6	0.0^{c}	0.0^{c}	0.4^{a}	0.2^{b}	0.02	<0.001
20:4n-3	0.0^{c}	0.0^{c}	1.7^{a}	0.9^{b}	0.07	<0.001
20:4n-6	0.0^{c}	0.0^{c}	1.9^{a}	1.0^{b}	0.08	<0.001
20:5n-3	0.0^{c}	0.0^{c}	39.0^{a}	20.4^{b}	1.68	<0.001
21:5n-3	0.0^{c}	0.0^{c}	1.6^{a}	0.9^{b}	0.07	<0.001
22:0	1.1^{c}	1.4^{a}	1.2^{bc}	1.3^{ab}	0.04	0.012
22:1 *cis*-11	0.0^{c}	0.0^{c}	1.7^{a}	0.9^{b}	0.08	<0.001
22:1 *cis*-13	0.3^{c}	0.3^{c}	0.9^{a}	0.6^{b}	0.03	<0.001
22:5n-3	0.0^{c}	0.0^{c}	4.1^{a}	2.2^{b}	0.18	<0.001
22:5n-6	0.0^{c}	0.0^{c}	0.6^{a}	0.3^{b}	0.03	<0.001
22:6n-3	0.0^{c}	0.0^{c}	24.7^{a}	12.9^{b}	1.07	<0.001
24:0	7.3	6.8	6.4	6.7	0.17	0.07
Total SFA	56.2^{d}	72.4^{c}	116^{a}	97.0^{b}	4.07	<0.001
Total MUFA	68.2^{c}	108^{b}	128^{a}	121^{ab}	4.4	<0.001
Total PUFA	106^{d}	287^{a}	197^{c}	247^{b}	6.5	<0.001
Total fatty acids	231^{b}	470^{a}	447^{a}	469^{a}	14.8	<0.001

[a–d]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (LFO).

[2]Zero values indicate fatty acids in feed ingredients were <0.005 mg/g of DM.

Ruminal Biohydrogenation

Flows of unsaturated fatty acids were less than their respective intake, indicating extensive hydrogenation in the rumen. Both FO and LFO increased ($P < 0.001$) the extent of *cis*-9 16:1 biohydrogenation compared with the control or LO (Table 8). Ruminal biohydrogenation of *cis*-9 18:1 and 18:2n-6 was the same across treatments, whereas all oil supplements increased ($P < 0.001$) the extent of 18:3n-3 biohydrogenation in the rumen (Table 8). Biohydrogenation of 18:3n-3 was more extensive ($P < 0.001$) on LO and LFO than FO. Furthermore, 20:5n-3 and 22:6n-3, unique to FO, were extensively hydrogenated in the rumen, the extent of which was marginally, but significantly, decreased ($P = 0.05$) for LFO compared with FO (Table 8).

Nutrient Digestibility

Inclusion of oils in the diet had no effect ($P > 0.05$) on ruminal or whole-tract apparent DM, OM, N, NDF, and starch digestibility coefficients (Table 9) or on the proportion of nutrients digested in the rumen (data not presented).

Table 4. Effect of linseed oil and fish oil in the diet on rumen fermentation characteristics in growing cattle

	Treatment[1]					
Item	Control	LO	FO	LFO	SEM	*P*-value
pH	6.51	6.48	6.57	6.53	0.055	0.71
Total VFA, m*M*	56.9	56.2	51.8	53.7	2.82	0.58
Molar proportion, mmol/mol						
Acetate	659[a]	649[a]	608[b]	638[a]	7.0	0.010
Propionate	188[c]	187[c]	237[a]	209[b]	4.4	<0.001
Butyrate	133	138	132	129	4.7	0.60
Isovalerate	16.0	22.3	19.5	24.8	4.92	0.65
Molar ratio						
Acetate:propionate	3.50[a]	3.49[a]	2.57[c]	3.06[b]	0.091	0.001

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (LFO).

DISCUSSION

Several studies have examined the potential of FO (Shingfield et al., 2003; Kim et al., 2008; Lee et al., 2008), LO (Loor et al., 2004; Doreau et al., 2009b), or linseed (Scollan et al., 2001; Gonthier et al., 2004b; Doreau et al., 2009b) in the diet to alter the supply of n-3 PUFA available for absorption in cattle. Furthermore, the effects of supplementing the diet with LO and FO on the flow of fatty acids at the duodenum have been examined in steers (Scollan et al., 2001) and sheep (Wachira et al., 2000; Chikunya et al., 2004), but there are no reports on the impact of feeding a combination of FO and LO on ruminal lipid metabolism in growing cattle.

Nutrient Intake and Digestion

Inclusion of FO, LO, or an equal amount of both oils in the diet at 3% of DM had no effect on DMI compared with the control in this experiment, which may, at least in part, be attributed to steers being fed at a restricted level of intake. Previous investigations have shown that incremental amounts of FO in the diet from 0 to 3.9% DM had no effect on DMI in steers offered grass (*Lolium* spp.) silage diets ad libitum (Keady and Mayne, 1999) or at restricted levels (Kim et al., 2008; Lee et al., 2008). In contrast, inclusion of FO at 2.4 to 3% DM has been reported to decrease the intake of growing cattle fed red clover (*Trifolium pratense*) silage (Lee et al., 2008) or maize silage-based diets (Shingfield et al., 2010b) at 90 to 95% of ad libitum intake. Reports on the impact of LO in the diet in growing cattle are limited, but the addition of linseed in the diet supplying about 3% oil has been reported to either decrease (Sutter et al., 2000) or have no effect on DMI (Scollan et al., 2001). In cows, LO at 2.6 to 3.0% diet DM has no effect on intake (Ueda et al., 2003; Doreau et al., 2009a), but at inclusion rates of 5.7% DMI was decreased (Martin et al., 2008).

Both LO and FO in the diet had no effect on ruminal DM, OM, NDF, or starch digestion. In earlier experiments, FO has been reported to decrease the extent of OM and NDF digestion in the rumen in cattle fed grass silage as the sole feed (Lee et al., 2008), but increase these variables when replacing calcium salts of palm oil fatty acids in the diet (Kim et al., 2008). In other experiments, FO was found to have no effect on the proportion of these nutrients digested in the forestomach in steers fed red clover silage (Lee et al., 2008) or maize silage-based diets (Shingfield et al., 2010b). Supplementing the diet with up to 3% of lipid in the form of LO (Ueda et al., 2003; Doreau et al., 2009a) or linseed (Sutter et al., 2000; Doreau et al., 2009a) did not alter or may improve (Gonthier et al., 2004a) ruminal or total tract digestibility coefficients, but at greater amounts (5.7% of diet DM), total NDF digestion was decreased (Martin et al., 2008). Overall, data are consistent with the extent to which FO or linseed lipids in the diet alter nutrient digestion being dependent on several factors including the amount of oil inclusion, composition of the basal diet, and DMI (Doreau et al., 2009a; Shingfield et al., 2010b).

Rumen Fermentation

Supplements of FO had no effect on rumen pH, but shifted rumen fermentation toward propionate at the expense of acetate with no change in molar proportions of butyrate. Earlier studies reported that FO has no major effect on rumen pH or fermentation characteristics in growing cattle fed grass silage or red clover silage as the sole feed (Lee et al., 2008), or diets containing grass silage (Keady and Mayne, 1999; Kim et al., 2008), but enhanced the ratio of glucogenic:lipogenic precursors in the rumen of steers fed maize silage-based diets (Shingfield et al., 2010b). It is probable that changes in rumen fermentation patterns are related to the effect of FO on nutrient digestion in the rumen and alterations in the relative abundance of specific microbial populations.

Inclusion of LO in the diet had no effect on rumen fermentation patterns compared with the control, consistent with previous reports in cattle (Doreau et al.,

Table 5. Effect of linseed oil and fish oil in the diet on the flow of nutrients at the duodenum in growing cattle

	Treatment[1]					
Flow, g/d	Control	LO	FO	LFO	SEM	*P*-value
DM	5,389	5,688	5,142	5,584	193.2	0.30
OM	4,483	4,749	4,360	4,585	186.9	0.55
NDF	1,484	1,546	1,448	1,587	73.1	0.58
Starch	472	430	445	482	60.6	0.92
N	224	218	188	204	8.0	0.08
Nonammonia N	219^{a}	213^{a}	182^{b}	199^{ab}	7.6	0.050
12:0	0.48	0.50	0.53	0.50	0.050	0.93
13:0	0.12	0.13	0.14	0.15	0.013	0.37
14:0	2.11^{c}	2.27^{c}	9.33^{a}	6.28^{b}	0.451	<0.001
15:0	2.59^{c}	2.97^{bc}	4.09^{a}	3.75^{ab}	0.226	0.011
15:0 *iso*	1.47^{b}	1.59^{b}	2.00^{a}	1.71^{ab}	0.097	0.039
15:0 *anteiso*	3.01	3.30	3.87	3.36	0.213	0.13
16:0	39.1^{c}	49.9^{b}	84.2^{a}	74.8^{a}	3.01	0.001
16:0 *iso*	1.16	1.66	0.93	1.73	0.277	0.22
3, 7, 11, 15-tetramethyl-16:0	0.27^{c}	0.36^{b}	0.48^{a}	0.47^{a}	0.026	0.004
16:1 total	0.67^{c}	0.81^{c}	10.9^{a}	5.71^{b}	0.892	<0.001
17:0	1.75^{b}	1.98^{b}	3.11^{a}	2.69^{a}	0.161	0.003
17:0 *iso*	0.68	0.71	1.00	1.06	0.104	0.09
17:0 *anteiso*	1.39	1.48	1.89	2.06	0.182	0.11
17:1 *cis*-8	0.0^{c}	0.0^{c}	0.14^{a}	0.08^{b}	0.010	0.001
18:0	157^{b}	273^{a}	51.4^{d}	123^{c}	9.04	<0.001
18:1 total *cis*	17.9^{b}	31.0^{a}	28.9^{a}	31.1^{a}	1.17	<0.001
18:1 total *trans*	24.5^{c}	84.4^{b}	108^{ab}	125^{a}	11.2	0.002
18:1 total	42.4^{c}	115^{b}	137^{ab}	156^{a}	12.0	0.003
CLA total	0.38^{b}	3.18^{a}	0.27^{b}	0.55^{b}	0.368	0.004
18:3n-3	1.57^{bc}	4.36^{a}	1.23^{c}	2.29^{b}	0.217	<0.001
20:0	2.68^{b}	3.22^{b}	2.86^{b}	5.62^{a}	0.351	0.003
20:1 *cis*-11	0.29^{b}	0.42^{b}	3.32^{a}	2.72^{a}	0.192	<0.001
20:2n-6	0.06^{c}	0.08^{c}	0.83^{a}	0.35^{b}	0.055	<0.001
20:3n-3	0.04^{b}	0.04^{b}	5.14^{a}	0.86^{b}	0.414	<0.001
20:3n-6	0.05^{b}	0.10^{b}	0.49^{a}	0.17^{b}	0.053	0.004
20:4n-6	0.09	0.20	0.11	0.08	0.040	0.24
20:5n-3	0.11^{b}	0.16^{b}	1.25^{a}	0.93^{a}	0.120	0.001
22:0	1.35^{c}	1.68^{b}	1.37^{c}	2.03^{a}	0.055	<0.001
22:1 *cis*-13	0.18^{c}	0.21^{c}	0.61^{a}	0.40^{b}	0.039	<0.001
22:2n-6	0.02^{b}	0.02^{b}	0.13^{a}	0.11^{a}	0.012	<0.001
22:4n-6	0.20^{b}	0.17^{b}	0.32^{a}	0.16^{b}	0.023	0.008
22:5n-3	0.15^{c}	0.17^{c}	2.62^{a}	0.80^{b}	0.175	<0.001
22:6n-3	0.07^{b}	0.08^{b}	0.97^{a}	0.78^{a}	0.117	0.003
23:0	0.29^{b}	0.31^{ab}	0.36^{a}	0.38^{a}	0.018	0.046
24:0	1.75	1.93	1.62	1.85	0.106	0.29
Total SFA	217^{b}	347^{a}	170^{c}	232^{b}	7.4	<0.001
Total MUFA	43.6^{c}	117^{b}	153^{ab}	165^{a}	12.5	0.002
Total PUFA	17.0^{b}	31.1^{a}	31.3^{a}	30.5^{a}	1.01	<0.001
Total n-3 PUFA	1.94^{c}	4.80^{b}	11.2^{a}	5.65^{b}	0.62	<0.001
Total fatty acids	278^{d}	495^{a}	354^{c}	428^{b}	12.3	<0.001

$^{a-d}$Within a row, means without a common superscript differ ($P < 0.05$).

[1]Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (LFO).

2009a). In other experiments, LO (Ueda et al., 2003) or linseed (Gonthier et al., 2004a) supplying 3 to 4.0% of additional lipid in the diet have been shown to increase molar proportions of propionate at the expense of acetate. Given that FO altered ruminal VFA, whereas LO had no effect, it appears that the changes in rumen fermentation to LFO are due to fish oil. Previous studies have demonstrated that the effects of FO and whole linseed in the diet on the concentration and proportions of major VFA in the rumen are not additive in cattle (Scollan et al., 2001) or sheep (Wachira et al., 2000; Chikunya et al., 2004).

Ruminal Biohydrogenation

Comparison of the intake and duodenal flow of fatty acids indicated a net difference of 47, 25, −93, and −41 g/d for control, LO, FO, and LFO, respectively. A positive contribution of bacterial lipid to total fatty acid flow at the duodenum on the control and LO treatments is consistent with 75 to 80% of ingested fatty acids being recovered at the duodenum in ruminants fed typical diets (Doreau and Ferlay, 1994; Schmidely et al., 2008). Earlier studies have shown that LO tends to decrease net lipid balance in the rumen and at greater

Table 6. Effect of linseed oil and fish oil in the diet on the flow of 16:1 and 18:1 fatty acids at the duodenum in growing cattle

	Treatment[1]					
Flow, g/d	Control	LO	FO	LFO	SEM	*P*-value
16:1 *cis*-9	0.54^{c}	0.57^{c}	3.67^{a}	1.94^{b}	0.276	<0.001
16:1 *trans*-6	0.0^{c}	0.0^{c}	0.13^{b}	0.24^{a}	0.020	<0.001
16:1 *trans*-7 + 8	0.0^{c}	0.0^{c}	0.90^{a}	0.34^{b}	0.104	0.003
16:1 *trans*-9	0.01^{c}	0.05^{c}	0.79^{a}	0.41^{b}	0.087	0.003
16:1 *trans*-10	0.0^{c}	0.0^{c}	1.11^{a}	0.48^{b}	0.130	0.003
16:1 *trans*-11	0.0^{c}	0.0^{c}	2.64^{a}	1.14^{b}	0.200	<0.001
16:1 *trans*-12	0.12^{b}	0.18^{b}	0.99^{a}	0.73^{a}	0.091	0.001
16:1 *trans*-13	0.0^{c}	0.0^{c}	0.70^{a}	0.44^{b}	0.060	<0.001
18:1 *cis*-9	12.8^{c}	22.2^{a}	16.9^{b}	22.1^{a}	1.00	0.001
18:1 *cis*-11	2.61^{c}	3.20^{bc}	8.26^{a}	4.94^{b}	0.650	0.003
18:1 *cis*-12	2.30^{b}	3.79^{a}	2.52^{b}	2.51^{b}	0.117	<0.001
18:1 *cis*-13	0.06^{c}	0.32^{b}	0.48^{ab}	0.55^{a}	0.047	0.001
18:1 *cis*-15	0.18^{b}	1.53^{a}	0.80^{ab}	1.08^{a}	0.224	0.027
18:1 *trans*-4	0.23^{b}	0.69^{a}	0.15^{b}	0.56^{a}	0.093	0.017
18:1 *trans*-5	0.16^{b}	0.42^{a}	0.11^{b}	0.42^{a}	0.042	0.003
18:1 *trans*-6 + 7 + 8	1.58^{b}	4.67^{a}	5.08^{a}	5.60^{a}	0.604	0.012
18:1 *trans*-9	1.09^{c}	2.84^{b}	4.45^{ab}	4.88^{a}	0.490	0.006
18:1 *trans*-10	2.05^{b}	3.91^{b}	19.2^{a}	6.44^{b}	2.45	0.010
18:1 *trans*-11	13.8^{c}	51.2^{b}	66.7^{ab}	82.9^{a}	8.87	0.007
18:1 *trans*-12	1.47^{c}	4.79^{b}	5.85^{b}	8.70^{a}	0.640	0.001
18:1 *trans*-13 + 14	1.01^{c}	4.47^{a}	2.68^{b}	4.77^{a}	0.263	<0.001
18:1 *trans*-15	1.40^{c}	5.76^{a}	3.25^{b}	6.25^{a}	0.356	<0.001
18:1 *trans*-16[2]	1.63^{c}	5.63^{a}	0.98^{c}	4.29^{b}	0.349	<0.001

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (LFO).

[2]Contains *cis*-14 18:1 as a minor component.

amounts can result in fatty acid flow at the duodenum being less than dietary intake (Loor et al., 2004; Doreau et al., 2009b). A net loss of fatty acids on the FO treatment is consistent with previous observations in growing cattle fed diets containing between 2 and 3% DM of FO (Kim et al., 2008; Lee et al., 2008; Shingfield et al., 2010b).

Supplementing maize silage-based diets with FO and LFO increased the flow of 20:5n-3 (mean recovery 3.21 and 4.56%, respectively) and 22:6n-3 (corresponding

Table 7. Effect of linseed oil and fish oil in the diet on the flow of 18:2 fatty acids at the duodenum in growing cattle

	Treatment[1]					
Flow, mg/d	Control	LO	FO	LFO	SEM	*P*-value
Nonconjugated 18:2						
cis-9,*cis*-12	12,061	11,471	8,703	8,682	1,587.6	0.37
trans-11,*cis*-15	772^{c}	6,728^{a}	4,451^{b}	7,872^{a}	656.3	0.001
trans-9,*trans*-12	202^{c}	2,880^{a}	1,504^{b}	2,914^{a}	331.4	0.003
Conjugated 18:2						
cis-9,*trans*-11	194^{b}	628^{a}	107^{b}	264^{b}	65.4	0.006
cis-12,*trans*-14	1.1	37.1	5.3	4.3	11.96	0.22
trans-8,*cis*-10	0.0^{b}	6.9^{a}	5.3^{ab}	9.9^{a}	1.78	0.038
trans-10,*cis*-12	16.5	24.2	29.4	12.1	3.96	0.08
trans-11,*cis*-13	30.2^{b}	1,194^{a}	7.9^{b}	96.2^{b}	227.0	0.026
trans-13,*cis*-15	0.0^{b}	55.0^{a}	0.0^{b}	1.4^{b}	9.23	0.013
trans-8,*trans*-10	7.6	12.4	22.4	14.3	6.22	0.46
trans-9,*trans*-11	54.2^{b}	163^{a}	55.8^{b}	79.9^{b}	22.0	0.038
trans-10,*trans*-12	27.3^{b}	57.5^{a}	14.4^{bc}	10.1^{c}	4.03	<0.001
trans-11,*trans*-13	34.9^{b}	690^{a}	11.2^{b}	26.6^{b}	44.9	<0.001
trans-12,*trans*-14	12.7^{b}	289^{a}	9.0^{b}	29.8^{b}	11.8	<0.001
trans-13,*trans*-15	0.0^{b}	21.3^{a}	0.0^{b}	0.0^{b}	0.52	<0.001

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (LFO).

Table 8. Effect of linseed oil and fish oil in the diet on the apparent ruminal biohydrogenation of unsaturated fatty acids in growing cattle

Biohydrogenation, g/g	Treatment[1]				SEM	*P*-value
	Control	LO	FO	LFO		
16:1 *cis*-9	0.490	0.440	0.810	0.820	0.0380	<0.001
18:1 *cis*-9	0.772	0.765	0.779	0.750	0.0146	0.57
18:2n-6	0.869	0.911	0.907	0.923	0.0165	0.22
18:3n-3	0.864[c]	0.972[a]	0.904[b]	0.973[a]	0.0048	<0.001
20:5n-3	—	—	0.968[a]	0.955[b]	0.0038	<0.001
22:6n-3	—	—	0.961[a]	0.941[b]	0.0062	0.001

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (LFO).

values 3.93 and 6.05%) at the duodenum, but the increases were marginal relative to the intake, indicating that these fatty acids were extensively hydrogenated in the rumen. Ruminal escape of 20:5n-3 and 22:6n-3 as a function of intake was less in steers fed FO than LFO, consistent with the extent of biohydrogenation of these fatty acids in the rumen increasing in direct relation to the amount of FO in the diet (Kim et al., 2008; Lee et al., 2008; Shingfield et al., 2010b). Both LO and LFO increased 18:3n-3 at the duodenum, but ruminal escape accounted for 2.81 and 2.73% of dietary intake, respectively. Oil supplements increased 18:3n-3 biohydrogenation in the rumen, but there was no difference in the extent of ruminal 18:3n-3 biohydrogenation between the LFO and LO diet. Extensive evaluation of published studies has shown that the relative proportion of ingested 18:3n-3 escaping the rumen is not directly related to the amount of 18:3n-3 in the diet (Doreau and Ferlay, 1994). Overall there was no evidence in this experiment to indicate that fatty acids in FO inhibited ruminal *cis*-9 18:1, 18:2n-6, or 18:3n-3 biohydrogenation or that LO altered the extent of ruminal biohydrogenation of 20:5n-3 and 22:6n-3 in growing cattle.

Inclusion of FO in the diet increased the flow of several *cis* and *trans* 16:1 isomers, consistent with previous studies in growing cattle (Shingfield et al., 2010b). Duodenal digesta in steers fed FO and LFO was devoid of 16:2n-4, 16:4n-1, and 16:4n-3 indicating that these fatty acids are at least partially hydrogenated in the rumen. Incomplete biohydrogenation of all *cis* C16 unsaturated fatty acids contained in FO may explain the appearance of *trans* 16:1 (Δ6–8,10,11,13) in duodenal digesta of steers fed FO and LFO.

Despite similar intakes of 18.2n-6 and 18:3n-3, FO increased the flow of *trans*-11,*cis*-15 18:2 and *trans*-9,*trans*-12 18:2 at the duodenum compared with the control. Earlier studies have also shown that FO causes dose-dependent increases in the amount of *cis*, *trans* 18:2, *trans*, *cis* 18:2, and *trans*, *trans* 18:2 biohydrogenation intermediates at the duodenum in growing cattle (Kim et al., 2008; Lee et al., 2008; Shingfield et al., 2010b). Studies in vitro have shown that *trans*-9,*trans*-12 18:2 is formed during incubations of 18:2n-6 with ruminal fluid (Jouany et al., 2007) and confirmed *trans*-11,*cis*-15 18:2 as an intermediate of ruminal 18:3n-3 biohydrogenation (Harfoot and Hazlewood,

Table 9. Effect of linseed oil and fish oil in the diet on apparent ruminal and whole-tract digestibility coefficients in growing cattle

Digestibility coefficient[1]	Treatment[2]				SEM	*P*-value
	Control	LO	FO	LFO		
Forestomach						
DM	0.393	0.348	0.377	0.368	0.0352	0.83
OM	0.445	0.404	0.422	0.432	0.0347	0.85
NDF	0.412	0.375	0.374	0.355	0.0337	0.69
Starch	0.760	0.770	0.764	0.764	0.0282	0.99
N	0.241	0.208	0.270	0.257	0.0471	0.81
Whole tract						
DM	0.710	0.697	0.725	0.722	0.0139	0.53
OM	0.729	0.715	0.747	0.740	0.0124	0.36
NDF	0.544	0.510	0.564	0.535	0.0208	0.40
Starch	0.943	0.945	0.952	0.937	0.0069	0.52
N	0.784	0.770	0.784	0.792	0.0124	0.66

[1]Digestbility coefficient calculated as intake-flow at the duodenum/intake.

[2]Refers to maize silage-based diets containing 0 (control) or 30 g/kg of DM of linseed oil (LO), fish oil (FO), or a mixture (1:1 wt/wt) of linseed oil and fish oil (LFO).

1988; Wąsowska et al., 2006; Jouany et al., 2007). Furthermore, FO causes *trans*-11,*cis*-15 18:2 and *trans*-11 18:1 to accumulate during incubations of ruminal fluid with 18:3n-3 (Chow et al., 2004; Wąsowska et al., 2006), indicating that 1 or more fatty acids in FO inhibit the reduction of *trans* 18:1 and 18:2 intermediates to 18:0. Incubations of pure fatty acid substrates with ruminal fluid have shown that 20:5n-3 and 22:6n-3 in FO inhibit the complete hydrogenation of C18 unsaturated fatty acids in vitro (AbuGhazaleh and Jenkins, 2004a,b; Wąsowska et al., 2006).

Supplementing the diet with FO resulted in an increase in *trans* 18:1 and a decrease in 18:0 at the duodenum. Several experiments have shown that irrespective of the composition of the basal diet, FO causes a dose-dependent increase in the flow of *trans* 18:1 and concomitant decrease in 18:0 at the duodenum in growing cattle (Kim et al., 2008; Lee et al., 2008; Shingfield et al., 2010b), effects that may involve alterations in total ruminal eubacteria and *Butyrivibrio* populations (Kim et al., 2008; Huws et al., 2010). Relative to the control and LO, FO in the diet increased *trans*-10 18:1 at the duodenum. Studies with pure cultures of ruminal *Butyrivibrio* species or mixed rumen microbes have shown that *trans*-10 18:1 can be formed by the reduction of *trans*-10,*cis*-12 CLA (McKain et al., 2010) or isomerization of *cis*-9 18:1 (Mosley et al., 2002). In the present experiment, increases in *trans*-10 18:1 at the duodenum to FO were not accompanied by greater flows of *trans*-10,*cis*-12 CLA, consistent with previous findings in lactating cows (Shingfield et al., 2003) and growing cattle (Lee et al., 2008).

Across all treatments *cis*-9,*trans*-11 was the major isomer of CLA at the duodenum, confirming earlier reports in cattle fed maize silage-based diets (Piperova et al., 2002; Doreau et al., 2009b; Shingfield et al., 2010b). Duodenal digesta was devoid of *trans*-7,*cis*-9 CLA, consistent with earlier reports in cattle fed a wide range of diets (Piperova et al., 2002; Shingfield et al., 2003; Lee et al., 2008). Supplements of FO had no effect on the flow of CLA isomers at the duodenum compared with the control. In previous investigations, FO has been reported to increase ruminal outflow of *trans*-7,*trans*-9 CLA, *trans*-8,*trans*-10 CLA, and *trans*-9,*trans*-11 CLA and decrease *cis*-12,*trans*-14 CLA accumulation in lactating cows fed diets containing grass silage (Shingfield et al., 2003) or in growing cattle offered red clover silage or grass silage (Lee et al., 2008). In steers fed maize silage-based diets, inclusion of FO from 0 to 1.6% was shown to result in marginal increases in *cis*-9,*trans*-11 CLA and *trans*-9,*trans*-11 at the duodenum, changes that were not evident when the amount of FO in the diet was further increased to 2.4% of DM (Shingfield et al., 2010b).

Inclusion of LO in the diet increased 18:0, *cis* 18:1 (Δ9,12,13,15), *trans* 18:1 (Δ4–9,11–16), *trans*-11,*cis*-15 18:2, *trans*-9,*trans*-12 18:2, and total CLA flow at the duodenum, which is in agreement with the effects of LO on duodenal fatty acid flow in cows fed diets containing variable proportions of grass hay (Loor et al., 2004) or maize silage (Doreau et al., 2009b). Numerous studies involving incubations with rumen inocula in vitro have established that the major pathway of 18:3n-3 metabolism involves initial isomerization to yield *cis*-9,*trans*-11,*cis*-15 18:3, which is transient and sequentially reduced via *trans*-11,*cis*-15 18:2 and *trans*-11 18:1 to yield 18:0 (Harfoot and Hazlewood, 1988; Wąsowska et al., 2006; Jouany et al., 2007). Increases in the flow of most fatty acids to LO can be explained by the incomplete hydrogenation of 18:3n-3 in the rumen (Jouany et al., 2007; Shingfield et al., 2010a), but LO also contains *cis*-9 18:1 and 18:2n-6 that contribute to the increase in the flow of specific intermediates (Jenkins et al., 2006; Jouany et al., 2007; Shingfield et al., 2010a) at the duodenum.

Inclusion of LO in the diet resulted in a greater flow of several positional and geometric isomers of CLA at the duodenum. Increases in the flow of *cis*-9,*trans*-11 CLA, *trans*-8,*cis*-10 CLA, *trans*-9,*trans*-11 CLA, and *trans*-10,*trans*-12 18:2 to LO can be attributed to an increase in 18:2n-6 intake (Wallace et al., 2007; Shingfield et al., 2008), whereas the appearance of 11,13 CLA, 12,14 CLA, and 13,15 CLA can be explained by incomplete hydrogenation of 18:3n-3 in the rumen (Jouany et al., 2007; Shingfield et al., 2010a). Previous studies have also reported that LO increases *cis*-9,*trans*-11 CLA, *trans*-8,*cis*-10 CLA, *trans*-11,*cis*-13 CLA, and *trans*-11,*trans*-13 CLA flow at the duodenum (Loor et al., 2004; Doreau et al., 2009b), whereas *trans*-11,*trans*-13 and *trans*-12,*trans*-14 are the main isomers of CLA in duodenal digesta of cattle fed 18:3n-3 rich forages as the sole feed (Doreau et al., 2007; Lee et al., 2008). Mechanisms explaining the formation of geometric isomers of 11,13 and 12,14 CLA are not known, but it has been suggested that *trans*-13,*cis*-15 CLA is formed from the reduction of *cis*-9,*trans*-13,*cis*-15 18:3 (Destaillats et al., 2005).

A major objective of this experiment was to establish whether supplementing the diet with LO as a source of 18-carbon unsaturated fatty acids in combination with FO, known to inhibit the reduction of *trans* 18:1 to 18:0, increases *trans*-11 18:1 flow at the duodenum compared with isoenergetic amounts of LO or FO. Increases in most 18-carbon biohydrogenation intermediates to LFO were comparable with or intermediate to responses to FO or LO, whereas duodenal flow of *trans*-11 18:1 was not increased on LFO compared with FO. In earlier studies, the relative proportion of *trans*-11 18:1 in total ruminal fatty acids was reported to be increased in cows fed FO and linseed compared with FO and a 18:0 enriched fat supplement (AbuGhazaleh et al., 2003). Inclusion of FO in high concentrate diets containing rapeseed oil was shown to increase *trans*-9 18:1 and *trans*-11 18:1 at the duodenum in steers, but not when the basal diet contained corn oil (Duckett and Gillis, 2010). Overall, data from this and previous experiments indicate that supplementing the diet with a mixture of marine lipids and plant oils or oilseeds

can be used to modify ruminal biohydrogenation and alter the flow of specific biohydrogenation intermediates available for absorption. There was no substantive evidence to indicate that LO and FO in the amounts tested in this experiment exert synergistic effects on ruminal fatty acid metabolism or accumulation of biohydrogenation intermediates in the rumen of growing cattle fed maize silage-based diets.

Changes in the flow of specific CLA isomers at the duodenum to LFO were intermediate relative to LO and FO in the diet, with the implication that fatty acids in FO do not directly interfere with the initial isomerization of 18:2n-6 or 18:3n-3 in the rumen or the reduction of conjugated biohydrogenation intermediates. *Butyrivibrio fibrisolvens* is thought to be the main bacterium responsible for the isomerization of 18-carbon PUFA and reduction of conjugated 18:3 isomers to *trans* 18:2 in the rumen (Lourenço et al., 2010). Both 20:5n-3 and 22:6n-3 in nonesterified form are known to inhibit the growth and isomerase activity of *B. fibrisolvens*, whereas FO has no effect (Wąsowska et al., 2006). Even though FO alters ruminal microbial ecology (Lee et al., 2008; Huws et al., 2010), no direct effect on the abundance of *B. fibrisolvens* in ruminal digesta has been reported. The lack of a clear association between the appearance of specific biohydrogenation intermediates with alterations in ruminal microbial community composition in vivo suggests other, as yet uncultivated, microbial species may be involved in the hydrogenation or hydration or both transformations of unsaturated fatty acids in the rumen (Lourenço et al., 2010; Huws et al., 2011).

In conclusion, LO, FO, or equal amounts of both oils in unprotected form altered the flow of dietary fatty acids and biohydrogenation intermediates at the duodenum of growing cattle. Ruminal escape of 18:3n-3, 20:5n-3, and 22:6n-3 was marginal relative to the intake from the diet with no evidence that FO fed alone or in combination with LO substantially alters the extent of 18-carbon PUFA biohydrogenation in the rumen. Inclusion of marine lipids or plant oils in the diet represent an effective nutritional strategy to increase the flow of specific biohydrogenation intermediates at the duodenum, but the potential to increase the supply of n-3 PUFA available for absorption in growing cattle appears limited.

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